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ORIGINAL ARTICLE

The effect of the GLP-1 analogue Exenatide on functional connectivity within an NTS-based network in women with and without obesity

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Summary

Objective

The differential effect of GLP-1 agonist Exenatide on functional connectivity of the nucleus tractus solitaries (NTS), a key region associated with homeostasis, and on appetite-related behaviours was investigated in women with normal weight compared with women with obesity.

Methods

Following an 8-h fast, 19 female subjects (11 lean, 8 obese) participated in a 2-d double blind crossover study. Subjects underwent functional magnetic resonance imaging at fast and 30-min post subcutaneous injection of 5 μg of Exenatide or placebo. Functional connectivity was examined with the NTS. Drug-induced functional connectivity changes within and between groups and correlations with appetite measures were examined in a region of interest approach focusing on the thalamus and hypothalamus.

Results

Women with obesity reported less hunger after drug injection. Exenatide administration increased functional connectivity of the left NTS with the left thalamus and hypothalamus in the obese group only and increased the correlation between NTS functional connectivity and hunger scores in all subjects, but more so in the obese.

Conclusions

Obesity can impact the effects of Exenatide on brain connectivity, specifically in the NTS and is linked to changes in appetite control. This has implications for the use of GLP-1 analogues in therapeutic interventions.

Keywords: Brain, GLP-1, hunger, obesity.

Introduction

Glucagon-like Peptide 1 (GLP-1) receptor agonists have shown to improve glucose control, decrease food intake, accelerate weight loss and increase satiety (1–3). Increases in post-prandial GLP-1 levels have also been implicated in the observed weight loss following bariatric surgery (1,4). Because of these effects, the role of GLP-1 in the regulation of food intake has been evaluated centrally in the brain particularly in the hypothalamus and brain stem where it affects appetite and food intake and peripherally in the ileum where it affects satiety (5). It has also been shown that peripherally administered GLP-1 can reach the brain via leaks in the blood brain barrier (5).

Glucagon-like Peptide 1 is primarily released from L cells in the distal gut, but it is also produced in the vagal nucleus of the solitary tract (NTS) (6), a brainstem region that plays a central role in the regulation of satiety. The NTS sends projections to the hypothalamus and interoceptive regions within the brain's homeostatic network (including thalamus and insular cortex) (7,8). Vagal signalling to the hypothalamus and thalamus is likely to play a

crucial role in mediating the impact of GLP-1 on ingestive behaviour (9), and abnormalities in this signalling have been implicated in the pathophysiology of obesity (10). While findings from rodent studies emphasize the important interactions between GLP-1 and NTS on body weight control and human studies have shown reduced appetite in human subjects in response to Liraglutide and other GLP-1 agonists, the central mechanisms of this molecule are incompletely understood (9,11).

Functional brain imaging techniques help bridge the gap between clinical observation and underlying neurobiological mechanisms. Measuring intrinsic brain oscillations during resting conditions has demonstrated altered functional connectivity of specific brain regions in clinical conditions including obesity (12). Altered NTS functional connectivity within the hypothalamus while looking at images of palatable food after consuming a caloric beverage has been previously demonstrated in women with obesity (13). These brain changes were correlated with alterations in taste ratings determined by a questionnaire, linking functional connectivity alterations in the NTS and hypothalamus to ingestive behaviour (13).

Based on previous observations of differential functional connectivity in individuals with obesity compared with those with normal weight, the current study aimed to measure the impact of the GLP-1 analogue Exenatide on the intrinsic functional connectivity of the NTS with other brain regions. By studying resting state activity in healthy female participants with normal weights and obesity, the current study aimed to test the following hypotheses: (i) Exenatide changes functional connectivity between NTS and the thalamus and hypothalamus; (ii) these drug-induced changes in functional connectivity are different in women with obesity compared with women with normal weight; and (iii) the alterations in NTS functional connectivity are correlated with behavioural measures related to eating such as calories consumed at a meal and subjective feelings of hunger.

Methods

Subject selection

Nineteen healthy female subjects, ages 18–40, were recruited through the G. Oppenheimer Center for Neurobiology of Stress and Resilience from flyers and website advertisements. The sample included 11 women with normal weight (mean age: 25.09 ± 4.83 years old) with a mean body mass index (BMI) = 21.38 kg/m^2 (range = 19.52–24.74 kg/m^2) and 8 women with obesity (mean age: 26.62 ± 7.63 years old) with mean BMI = 34.08 kg/m^2 (range = 30.72–37.56 kg/m^2). All subjects were right-handed, pre-menopausal and in the follicular phase of the menstrual cycle by self-report and classified as healthy after a clinical assessment that included a modified Mini-International Neuropsychiatric Interview Plus 5.0 (14), a brief structured interview for major Axis I psychiatric disorders in Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (15) and International Statistical Classification of Diseases 10th Revision (16). Further exclusion criteria included pregnancy, substance abuse, tobacco dependence, evidence of cardiovascular, gastrointestinal, hepatic, neurologic or psychiatric illness, diabetes, high blood glucose levels (<200 mg/dL), strenuous exercise (more than 8 h/week) and any eating disorders. Subjects were also excluded if they were currently on medication such as analgesics or antidepressants, had used any diet aids in the last month, had undergone any bariatric surgeries, were not able to undergo an MRI or could not eat one of three meal options. Throughout the study, subjects were asked to report any feelings of nausea.

The subjects provided written informed consent and all procedures were reviewed and approved by the UCLA Medical Institutional Review Board.

Study paradigm

The subjects came in for three visits, a screening visit to determine their eligibility followed by two nearly identical MRI visits where they received either the drug or saline placebo injection in a randomized, double-blinded fashion. All scanning began in the morning after at least 8 h of fasting (the subjects did not eat breakfast). Blood glucose levels were determined to be within the range of 65–126 mg/dL. Brain scans were obtained at a pre-injection baseline and at 25 min after drug/placebo administration. Blood samples were obtained at fasting, before the drug/placebo injection and at 25, 35 and 60 min after drug administration. Scores for hunger/satiety levels using a 100-point visual analogue scale were obtained at the same time as the blood draws. After scanning, the subjects were taken to a general clinical research area and given a pre-selected 1000 calorie meal composed of 30% fat, 20% protein and 50% carbohydrates and were instructed to 'eat until you are full'. Meals were measured before and after consumption to determine calories consumed.

The basic protocol is reflected in Figure 1.

MRI protocol

Before placement into the scanner, the subjects completed the Hospital Anxiety and Depression questionnaire (17). Following placement in the scanner, the subjects

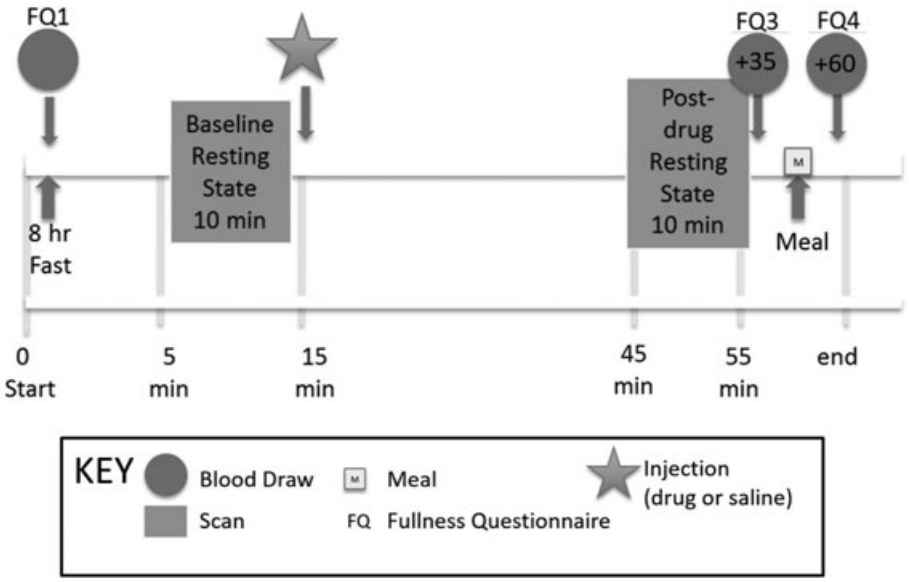

Figure 1 Basic scan day protocol. Subjects were brought in after at least 8 h of fasting. Scans were done both pre-subcutaneous and post-subcutaneous injection of either drug or saline placebo. After scanning, subjects were given a meal. Blood draws and questionnaires were given before scanning and 35 and 60 min after injection.

underwent a 3-min alignment true fast imaging with steady-state free precession (TRUFI) scan before a baseline 10-min resting state scan during which they were asked to lie still with their eyes closed and instructed not to fall asleep. After the resting scan, the subjects were removed from the scanner to receive either a subcutaneous injection of 5 μg of Exenatide or a 0.5-cc injection of saline (placebo) in the deltoid (upper arm) area. After the injection, the subjects were moved back into the scanner for a 9-min standard T1-weighted magnetization-prepared rapid acquisition gradient echo structural scan followed by a 10-min resting state scan that was completed 25–30 min after the injection.

Resting state data acquisition and pre-processing

Functional MRI resting state scans were acquired on a Siemens 3 Tesla Trio scanner. The 10-min scans were acquired with an echo planar sequence with the following parameters: TE = 28 ms, TR = 2,000 ms, flip angel = 77°, FOV = 220 mm, slices = 40, slice thickness = 4.0 mm. Slices were obtained with whole-brain coverage.

Image processing and data analysis were performed by using Statistical Parametric Mapping 8 (SPM8) software (Wellcome Department of Cognitive Neurology, London). Processing was done through the SPM toolbox, Data Processing Assistant for Resting-State fMRI (18). Data were slice-time, motion corrected, and nuisance covariate regression was preformed to minimize physiological noise by using six head motion parameters, white matter signal and cerebrospinal fluid signal. Data were spatially normalized to the Montreal Neurological Institute template by using the magnetization-prepared rapid acquisition gradient echo structural scan, which was acquired with the following parameters: TE = 3.26 ms, TR = 2,200 ms, slices = 176, slice thickness = 1.0 mm, voxel size = 1 × 1 × 1 mm. Spatial smoothing with a 4-mm^3 Gaussian kernel occurred after calculation of connectivity maps (18).

Blood sample processing

Blood samples were sent to outside laboratories for processing. Tamdem Labs California used an enzyme-linked immunosorbent assay to determine plasma concentration of Exenatide (19,20).

Data analyses

Clinical/behavioural characteristics

Analyses of clinical characteristics (age, BMI, education, anxiety and depression), behavioural measures (calorie consumption and fullness questionnaires) and blood levels of Exenatide were performed in SPSS 22 (21). Clinical characteristics were evaluated by using *t*-tests to compare group means (lean; obese). Anxiety and depression scores were compared separately for each day (screening day, drug day and placebo day). Levels of

Exenatide were compared between each group (lean; obese) by both day (drug; placebo) and time post injection (35 min; 60 min). In order to determine calorie consumption, a general linear model was run looking at group (lean: obese) × day (drug; placebo) by using blood levels of Exenatide as a covariate. Behavioural measures from the fullness questionnaire were analysed with ANOVAs looking at group (lean; obese) × time (baseline; 35 min post injection, 60 min post injection) and changes over time (i.e. 60 min post injection > 35 min post injection) for changes between days (drug day − placebo day).

Seed-based functional connectivity of the NTS

Using MarsBar (22), an NTS seed cluster was defined by a 4-mm sphere around the peak NTS coordinate determined by Kilpatrick *et al*. (13). Left and right clusters were defined separately. The functionally defined NTS cluster is simply referred to as the NTS in the Results and Discussion sections. Fisher transformed maps of the bivariate correlation between the NTS seed time course, and all other voxels were created by using Data Processing Assistant for Resting-State fMRI (18) for the left and right NTS. Based on significant results from Kilpatrick *et al*., this was done for a specific low-frequency band (0.01–0.027 Hz) (13,23). The resulting functional connectivity maps were analysed in a flexible factorial general linear model in SPM8 to compare group data (obese; lean) × day (drug; placebo) × scan (pre-injection; post injection). The order of the drug day was included as a factor in the model to account for any order effects. Age and blood levels of Exenatide were used as covariates. Using a region of interest approach similar to other studies (12,24), contrasts were performed to identify regions with altered connectivity with the NTS before or after the drug versus placebo, looking at both within-group and between-group differences. Anatomically based ROIs were created by using the Wake Forest University PickAtlas tookbox in SPM8. Hypothalamus and thalamus ROIs were chosen based on previous literature, defining them as regions associated with the NTS in hunger and satiety signalling (10). Images were thresholded at $p = 0.001$, and uncorrected and small volume corrections were applied to determine significance at $p < 0.05$, corrected for family wise error rate. False discovery rate was applied to control for the type I error inherent in testing multiple ROIs (25,26). Cluster size was limited to Ke > 3.

Functional connectivity correlations with behaviour

A multiple regression model in SPM8 was used to determine group differences in the association of calorie consumption and fullness questionnaire scores with functional connectivity of the NTS seed regions and designated ROIs described in the preceding texts. Blood levels of Exenatide, age and order of the drug day were used as covariates. The questionnaire responses selected for the correlational analysis were those that showed significant group differences in the behavioural SPSS analyses, which related to hunger. Contrast images were created to identify drug-induced changes in the correlation between functional connectivity and behavioural measure both within and between groups, as well as differences at the pre-injection baseline. Images were thresholded at $p = 0.001$, and uncorrected and small volume correction was employed to determine significance of the region of interest at $p < 0.05$ family wise error with additional false discovery rate correction to account for multiple comparisons (25,26). Cluster size was limited to Ke > 3.

Results

Clinical characteristics

There were no statistically significant group differences in age or any clinical variable other than BMI (Table 1). With all subjects in the clinically normal range, the two groups did not differ on any visit in anxiety symptom scores (screening: $F = 3.46$, $p = 0.080$; placebo day: $F = 1.233$, $p = 0.282$; drug day: $F = 3.28$, $p = 0.088$) or depression symptom scores (screening: $F = 4.139$, $p = 0.058$; placebo day: $F = 0.370$, $p = 0.552$; drug day: $F = 0.306$, $p = 0.558$; Table 1).

Exenatide levels

Both groups showed significantly higher levels of Exenatide in blood on the drug day compared with placebo day at all time points ($p < 0.001$; Table SS1 and Figure SS1). During the drug day, the lean group had higher plasma levels of Exenatide than the obese group both at 35 min (161 vs. 97.5 pg/mL), lean versus obese ($p = 0.004$) and 60 min (238.3 vs. 147.5 pg/mL, lean vs. obese $p = 0.001$) after injection, but both groups showed an increase of the drug between those times ($p = 0.007$). Due to the pharmacokinetic value differences between the groups, all subsequent analyses used the level of Exenatide in the blood as a covariate.

Glucose levels

All subjects combined on the drug day showed a decrease in mean blood glucose levels at both 35 min

Table 1 Subject clinical characteristics

	Lean $N = 11$	Obese $N = 8$	F	p-value
Age (year)	Mean: 25.09 Range: 21–36 SD: 4.83	Mean: 26.62 Range: 19–39 SD: 7.63	−0.538	0.598
BMI (kg/m^2)	Mean: 21.38 Range: 19.52–24.74 SD: 1.92	Mean: 34.08 Range: 30.72–37.56 SD: 2.35	168.54	<0.001**
Education	Mean: 4.55 SD: 2.34	Mean: 4.00 SD: 1.78	0.305	0.588
HAD Anxiety				
Screening	Mean: 2.91 SD: 3.21	Mean: 5.50 SD: 2.67	3.46	0.080
Placebo day	Mean: 3.00 SD: 4.05	Mean: 4.75 SD: 2.12	1.233	0.282
Drug day	Mean: 2.54 SD: 3.59	Mean: 5.00 SD: 1.51	3.28	0.088
HAD Depression				
Screening	Mean: 0.727 SD: 1.01	Mean: 1.87 SD: 1.46	4.139	0.058
Placebo day	Mean: 1.09 SD: 2.21	Mean: 1.63 SD:1.30	0.370	0.552
Drug day	Mean:1.18 SD: 2.56	Mean: 1.75 SD: 1.58	0.306	0.588

BMI, body mass index; HAD, Hospital Anxiety and Depression. p-values significant: 0.05* and 0.01**.
Education scoring: 1 = 8th grade or less; 2 = Some high school; 3 = High school graduate; 4 = Some college; 5 = College graduate; 6 = Any post-graduate work.

(77.35 vs. 86.56 mg/dL) and 60 min (75.87 vs. 86.56 mg/dL) after Exenatide injection compared with the placebo day ($p < 0.001$). Both lean and obese groups showed a significant reduction in mean blood glucose levels on the drug day between baseline and both 35 and 60 min post injection [lean baseline (83.8 mg/dL) vs. 35 min (75.90 mg/dL), $p = 0.026$; lean baseline vs. 60 min (73.78 mg/dL), $p = 0.030$; obese baseline (90.57 mg/dL) vs. 35 min (79.43 mg/dL), $p = 0.007$; obese baseline vs. 60 min (78.57 mg/dL), $p = 0.004$]. There were no significant differences between lean and obese groups (Figure SS2).

Hunger and appetite measures

There were no statistically significant group differences in calorie consumption on drug (468.21 vs. 620.25 kcal $p = 0.109$, $F = 1.27$) or placebo (569.10 vs. 652.81 kcal $p = 0.275$, $F = 2.86$) days. However, trends suggest that both groups consumed less calories on the drug day and that the obese group consumed more calories on both days than the lean group (Figure 2).

Regarding hunger scores, the only significant change was seen in the obese group on the day of the Exenatide injection (versus the placebo injection) when they reported to be less hungry than the lean group 35 min after drug injection (8.64 vs. −5.00; $p = 0.035$) and have a greater decrease in hunger scores after eating the

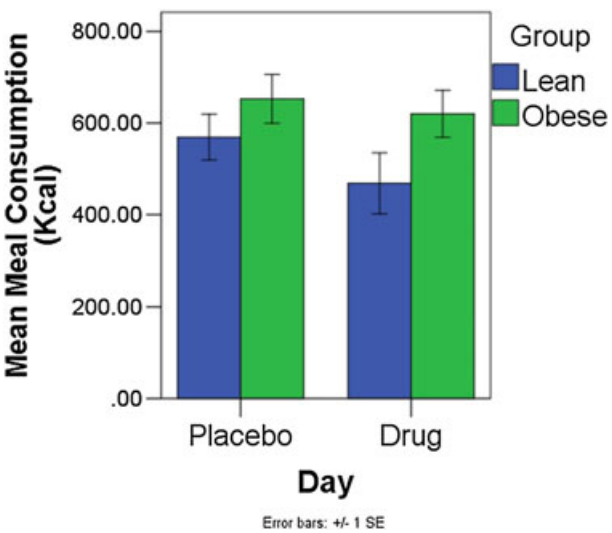

Figure 2 Calorie consumption of lean versus obese subjects of a post-scan meal. Trends show that both lean and obese groups consumed fewer calories on the day of the exenatide injection versus the placebo day and that the obese group consumed more calories on both days.

provided meal (7.27 vs. −10.00; $p = 0.025$). No significant group differences in hunger were observed before injection (Table 2). No group differences were observed for satiety levels.

Table 2 Fullness questions

Q: How hungry do you feel?			
Group	Time point	Mean	SD
Lean	Baseline	0	29.07
	+35 min	8.63	16.14
	+60 min	2.73	16.33
	+35 > baseline	8.64	31.71
	+60 > baseline	2.73	37.77
	+60 > +35 min	7.27	18.08
Obese	Baseline	−8.75	36.03
	+35 min	−5.00	5.34
	+60 min	−2.50	3.78
	+35 > baseline	3.75	38.89
	+60 > baseline	6.25	34.10
	+60 > +35 min	−10.00	9.64
Group difference	Time point	*F*	*p*-value
Lean vs. obese	Baseline	0.344	0.565
	+35 min	5.221	0.035
	+60 min	0.777	0.390
	+35 > baseline	0.091	0.766
	+60 > baseline	0.044	0.837
	+60 > +35 min	6.00	0.025
Q: How full do you feel?			
Group	Time point	Mean	SD
Lean	Baseline	5.45	13.62
	+35 min	−4.55	16.35
	+60 min	−3.64	13.80
	+35 > baseline	−10.00	19.34
	+60 > baseline	−9.09	18.00
	+60 > +35 min	−1.36	26.00
Obese	Baseline	−0.625	21.78
	+35 min	7.50	10.35
	+60 min	−3.12	6.51
	+35 > baseline	8.12	22.67
	+60 > baseline	3.75	21.84
	+60 > +35 min	14.37	23.37
Group difference	Time point	*F*	*p*-value
Lean vs. obese	Baseline	0.555	0.467
	+35 min	3.338	0.085
	+60 min	1.635	0.218
	+35 > baseline	3.52	0.078
	+60 > baseline	1.97	0.178
	+60 > +35 min	1.844	0.192

Asked the question 'How hungry do you feel?', the obese group reported to be less hungry 35 min after Exenatide injection versus placebo. The obese group also had a greater decrease in hunger on the drug day (versus placebo day) after eating the meal (vs. before the meal). No group differences were observed with the question 'How full do you feel?' All mean scores are drug day minus placebo day.

Functional connectivity of the NTS

No differences in functional connectivity were observed at baseline between day and group. After Exenatide administration, in comparison with the placebo, the obese group demonstrated increased functional connectivity of the left NTS with the left thalamus and left hypothalamus. No drug-induced connectivity change was observed in the lean group. For the right NTS seed, the obese group, compared with the lean group, showed greater functional connectivity with the left thalamus after drug administration versus the placebo (Table 3 and Figure 3).

NTS functional connectivity and behavioural correlates

Calorie consumption

For all subjects combined, there was a significant increase in the correlation between calorie consumption and functional connectivity of the right NTS with the right hypothalamus following Exenatide administration (versus placebo; Table 4A and Figure 4A). No group differences of significant correlations were observed before or after the drug injection. No subject consumed the entire meal.

Hunger ratings

At baseline, the lean group showed a statistically greater correlation between hunger scores and functional connectivity of the right NTS with the left thalamus [(−10 −16 16), $p = 0.004$, Ke = 87, $z = 4.69$] than the obese group. After Exenatide injection (versus placebo), all subjects combined demonstrated a positive correlation between subjective feelings of hunger and functional connectivity of the right NTS with the right hypothalamus. The subjects with obesity had a statistically greater correlation between the hunger ratings and functional connectivity of the right NTS with the left thalamus compared with subjects with normal weight. The subjects with obesity showed a correlation between hunger scores and functional connectivity of the left NTS with the left thalamus (Table 4B and Figure 4B–D). No correlations were seen between NTS connectivity and satiety scores.

Discussion

Exenatide administration, in comparison with placebo, resulted in increased NTS functional connectivity with the thalamus and hypothalamus in subjects with obesity. Specifically, an Exenatide-induced increase in NTS

Table 3 Impact of Exenatide on NTS functional connectivity

Contrast	Lean	Obese	Obese > lean	Lean > obese	Lean + obese
Region					
L NTS functional connectivity					
L HYP					
Coordinate		(−6 −4 −12)			
Cluster (Ke)		5			
p-value		0.026*			
z-score		3.38			
R HYP					
Coordinate					
Cluster (Ke)					
p-value					
z-score					
L thalamus					
Coordinate		(−20 −36 8)			(−14 −26 16)
Cluster (Ke)		46			13
p-value		0.028*			0.055
z-score		4.86			3.77
R thalamus					
Coordinate		(10 −24 12)	(6 −36 4)		
Cluster (Ke)		7	13		
p-value		0.054	0.108		
z-score		3.79	3.56		
R NTS functional connectivity					
L HYP					
Coordinate					
Cluster (Ke)					
p-value					
z-score					
R HYP					
Coordinate					
Cluster (Ke)					
p-value					
z-score					
L thalamus	(Decreased functional connectivity)				
Coordinate	(−18 −24 4)	(−10 −16 18)	−10−16 16)		
Cluster (Ke)	14	8	51		
p-value	0.125	0.140	0.022*		
z-score	3.51	3.47	4.51		
R thalamus					
Coordinate			(8 −8 12)		
Cluster (Ke)			32		
p-value			0.022*		
z-score			4.47		

Using the NTS as a seed, Exenatide administration resulted in greater functional connectivity in the obese group for both right and left seeds in a low-frequency band (0.01–0.027 Hz) for hypothalamus and thalamus ROIs. For the left NTS, this change was not seen in the lean group and for the right NTS; this change was greater in the obese than the lean group. Combining groups, greater functional connectivity was observed in the left NTS. L, left; NTS, nucleus of the solitary tract; R, right.
*p-value observed after correction for multiple comparisons.

functional connectivity was seen in the obese group only between the left NTS with the left hypothalamus and thalamus. For the right NTS, the functional connectivity with the left thalamus was statistically greater in the obese group when compared with the lean group.

The brainstem, specifically the NTS, and the hypothalamus are regions associated with regulation of food intake and ingestive behaviour (3). The bidirectional connection between these two regions is involved in integrating peripheral signals from hormones in the regulation

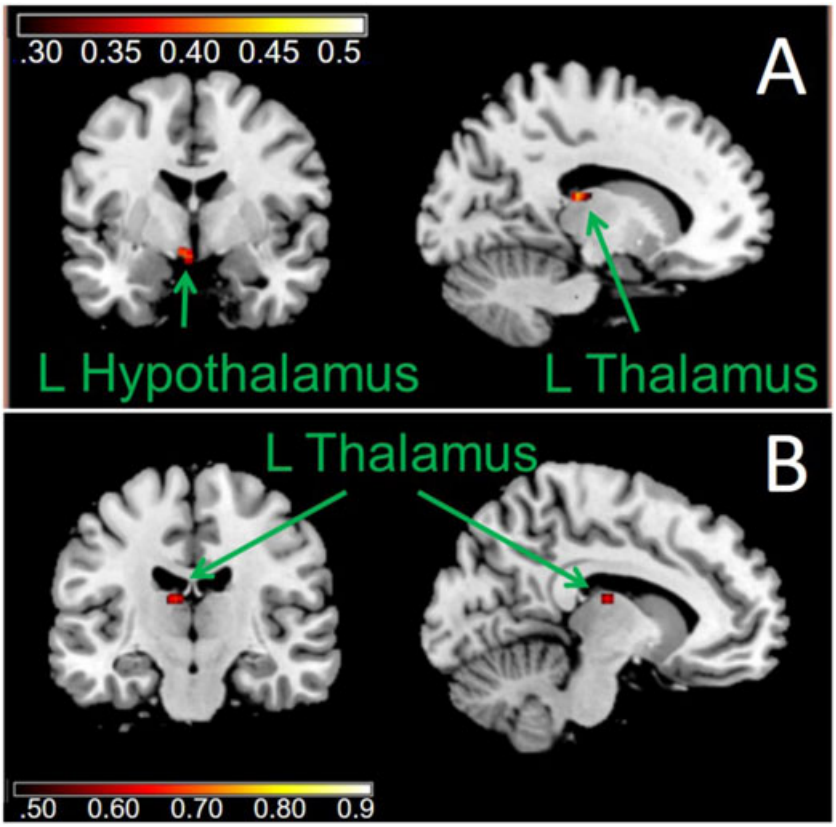

Figure 3 Functional connectivity analysis of nucleus of the solitary tract (NTS) with thalamus and hypothalamus in obese compared with lean. (A) The obese group showed increased left NTS functional connectivity with the thalamus and the hypothalamus and (B) compared with the lean group showed increased right NTS functional connectivity with the left thalamus.

of satiety (3), and differences in hypothalamus connectivity between individuals with obesity compared with those with normal weights have been shown to be reduced after successful bariatric surgery (27). This increased connectivity in response to the drug suggests an Exenatide-induced alteration of satiety signalling in individuals with obesity. Previous reports have suggested a decreased sensitivity to GLP-1 in obesity and have connected reduced endogenous GLP-1 and reduced GLP-1 receptor activation to weight gain (28). Imaging studies have linked increased receptor availability to increased functional connectivity in the brain (29). An exogenous GLP-1 agonist might be working to overcome a decreased sensitivity in obesity, and the Exenatide-induced increase in functional connectivity observed in this study is more so in the obese group could be reflective of the drug's ability to increase GLP-1 receptor activity in a depressed system.

This study also showed that after Exenatide injection, the obese group demonstrated significantly greater functional connectivity between the NTS and the thalamus compared with the lean group. Regions of the thalamus, particularly the paraventricular area receive input from the NTS, and these inputs are thought to play a role in homeostatic regulation of food intake (30). A greater increase in functional connectivity with the thalamus in the obese group could indicate that Exenatide may modulate homeostatic regulation of food intake differently in this group compared with more lean individuals.

As previously reported, the behavioural results of this study confirmed that Exenatide has an impact on feeding behaviours, showing changes in both calorie consumption and hunger levels in both obese subjects with obesity and subjects with normal weight (2,3).

Despite the greater reduction in their hunger scores after Exenatide, the subjects with obesity consumed more calories than the subjects with normal weights on both the Exenatide test day and placebo day. This could point to altered satiety signal processing as suggested by several studies in animals and humans that have shown obesity-related resistance to satiety signals such as leptin, insulin and GLP-1 at brain areas associated with eating control including the hypothalamus (28,31,32). It is conceivable that the administration of

Table 4 Exenatide-induced functional connectivity correlations with appetite measures

A. NTS functional connectivity and calorie consumption

Group	Region	Coordinate (*x*,*y*,*z*)	Cluster value (K)	*p*-value (family wise error)	*z*-score
L NTS functional connectivity correlation with calorie consumption					
Obese	L thalamus	(−20 −36 8)	25	0.051	3.74
	R thalamus	(24 −26 16)	8	0.144	3.39
Obese +	L thalamus	(−18 −36 10)	16	0.058	3.69
Lean	R thalamus	(24 −24 16)	5	0.200	3.27
R NTS functional connectivity correlation with calorie consumption					
Obese	R thalamus	(0 −2 4)	20	0.126	3.45
Obese >	L thalamus	(−8 14 16)	16	0.078	3.38
Lean	R thalamus	(6 −6 12)	15	0.119	3.47
Obese +	L HYP	(2 −4 8)	11	0.008*	3.69
Lean	L thalamus	(0 −2 2)	9	0.030*	3.90
	R thalamus	(0 −2 2)	32	0.064	3.90

B. NTS functional connectivity correlation with hunger ratings

Group	Region	Coordinate (*x*,*y*,*z*)	Cluster value (K)	*p*-value (family wise error)	*z*-score
L NTS functional connectivity correlation with hunger					
Obese	L thalamus	(−20 −36 8)	69	0.024*	4.20
	R thalamus	(24 −26 16)	17	0.054	3.94
Obese +	L thalamus	(−20 −34 10)	36	0.062	3.67
Lean	R thalamus	(24 −24 16)	9	0.107	3.50
R NTS functional connectivity correlation with hunger					
Lean (decreased correlation)	L thalamus	(−16 −24 4)	13	0.113	3.48
Obese	L thalamus	(−12 −18 18)	23	0.048	3.76
Obese >	L thalamus	(10 −16 16)	75	0.002*	4.76
Lean	R thalamus	(8 −8 12)	17	0.100	3.54
Obese +	L HYP	(4 −2 −6)	4	0.022*	3.39
Lean	R thalamus	(4 −4 −2)	16	0.083	3.60

All contrasts significant at p 0.001 uncorrected for drug–placebo, RSS3-RSS1 unless otherwise noted.
Functional connectivity of the nucleus of the solitary tract (NTS) seed with the hypothalamus and thalamus were observed to be correlated with behavioural measures as a result of Exenatide administration. (A) Calorie consumption was observed to be correlated with NTS functional connectivity in the obese group only for the left NTS seed and in both groups combined for the right NTS seed. (B) Subject hunger ratings were observed to be correlated with NTS functional connectivity in the obese group for the left NTS seed and in the obese group more significantly than in the lean group in the right NTS. L, left; NTS, nucleus of the solitary tract; R, right.
*p-value observed after correction for multiple comparisons.

Exenatide can overcome a baseline obesity-related resistance to GLP-1 satiety signals in women with obesity, whereas Exenatide in the women with normal weights may have a more immediate and dramatic impact on calorie consumption. The observed difference in hunger levels in the subjects with obesity might also, in part, be attributed to other factors associated with adiposity and obesity (33).

When all subjects were combined, a stronger correlation between calorie consumption and functional connectivity of the right NTS with the left hypothalamus was observed after Exenatide injection. Exenatide has been shown to increase hypothalamic connectivity with the rest of the brain in obese non-diabetic male subjects while the subjects were viewing pictures of food (34). This study suggests an Exenatide-induced strengthening of the relationship between NTS connectivity with the hypothalamus and calorie consumption that can be observed independent of exposure to visual food cues.

All subjects combined also showed a stronger Exenatide-induced correlation between subject hunger ratings and functional connectivity between the right NTS and right hypothalamus. The NTS is a known relay for vagal information from the gut and projects to the hypothalamus, which, in turn, communicates with emotion-regulating and higher cortical centres to generate sensations of hunger. These study results suggest the Exenatide-induced functional connectivity between these regions plays a role in subjective feelings of hunger, regardless of BMI.

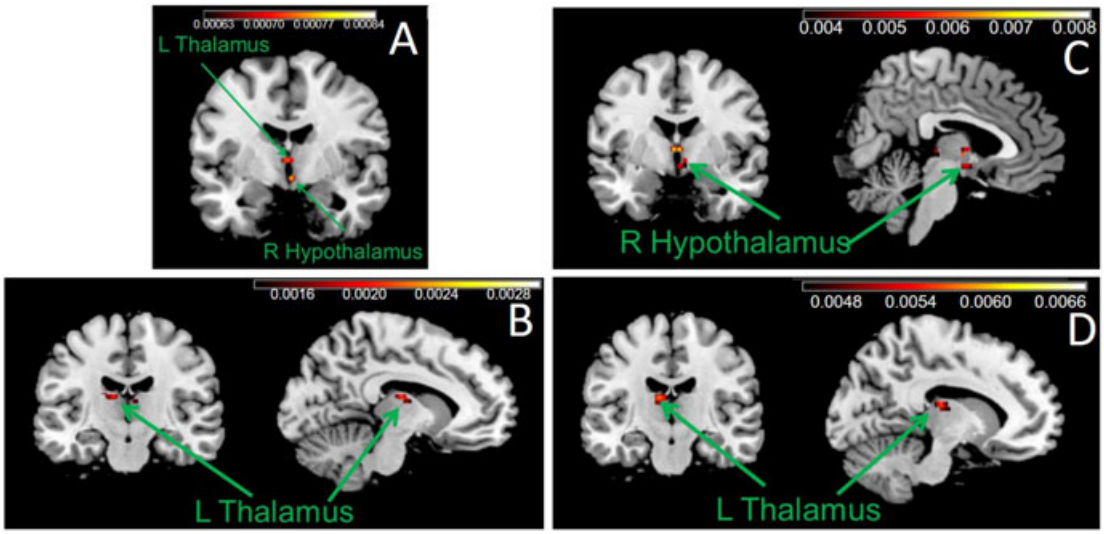

Figure 4 Nucleus of the solitary tract (NTS) functional connectivity correlation analysis with calorie consumption and hunger. (A) All subjects together showed a positive correlation between calories consumed of a meal and functional connectivity of the right NTS with the right hypothalamus and left thalamus. (B) At baseline before scanning, the lean group showed a greater correlation between hunger and right NTS functional connectivity with the left thalamus. After exenatide injection (versus placebo), (C) all subjects combined had a correlation between hunger and right NTS functional connectivity with the right hypothalamus and (D) the obese group had a greater correlation between hunger and right NTS functional connectivity with the left thalamus.

At baseline, before Exenatide administration, there was a stronger correlation between subjective hunger and functional connectivity of the right NTS and left thalamus in the lean compared with the obese group. However, after Exenatide administration, the obese group demonstrated a stronger relationship between hunger and functional connectivity of the NTS with the left thalamus, suggesting the drug's greater impact in strengthening the perhaps depressed engagement of an NTS-based network with feelings of hunger in the obese group.

Limitations

The current results, obtained in a relatively small sample, will require validation in a larger sample. Several studies have reported sex-related brain differences in obesity and eating behaviour, indicating that women with obesity have a greater brain response to food in regions associated with emotional processing and motivation (35). Larger studies would need to be performed to examine potential resting state differences between men and women.

There were also several measurements not collected that would have helped refine the results of this study. Resting state data may be influenced by factors beyond measures used in this study such as gastric emptying or other satiety hormones. Although the focus of this study was on analyses using a frequency band less influenced by physiological noise, autonomic functions have been demonstrated to effect resting state information (36). Although we asked subjects to report any feelings of nausea and none did, nausea is a known side effect of GLP-1 agonists and the NTS also plays a role in feelings of nausea and could impact other brain changes in this region (37). Also, although the varying levels of blood Exenatide were considered for these analyses, future studies would benefit from taking a weighted dosage of Exenatide based on body fat composition or body weight.

Although BMI is the current clinically used measure of obesity, it has been argued that other measures such as visceral fat or waist circumference may be better measures (38,39). This study also did not take into account any potential individual variants in the causes of obesity. Subsequent studies might benefit from examining factors such as food addiction, genetics, family history and stress to determine if there are any such measures that might differentiate the impact of Exenatide on brain connectivity results.

Summary and clinical implications

The release of several satiety hormones including GLP-1 is stimulated by nutrient ingestion and modulates the response of the central nervous system to food intake. GLP-1 stimulation, both in the periphery and within the brain, can impact the interpretation of hunger and satiety signalling. An imbalance or misinterpretation of these signals is thought to play a role in obesity (10). While several

studies examined the impact of GLP-1 analogues like Exenatide on stimulated brain responses, few have examined the impact of the drug on the resting brain. The current study demonstrates that following the acute administration of Exenatide, female subjects with obesity demonstrate a statistically significant decrease in the subjective feeling of hunger, and an associated increase in functional connectivity of the NTS, with the hypothalamus and thalamus, regions associated with eating behaviours. Exenatide also increased the positive correlation between the NTS-based functional connectivity and subjective feelings of hunger in all subjects, with a significantly greater impact in the obese group. As clinical trials are being conducted by using GLP-1 analogues as a potential weight loss treatment (9,40), the study of these hormones and their impact on the brain in obesity and obesity reduction is key to a complete understanding of the physiological mechanisms and potential effectiveness in the clinic.

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Conflict of interest

No conflicts of interest exist.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1: Post-injection PK values. Both lean and obese groups had significantly greater levels of Exenatide in the blood on the day of Exenatide injection compared with placebo injection. They also both had greater levels of Exenatide 60 min post injection versus 35 min post injection. Comparing groups, the lean group had greater levels of Exenatide in their bloodstream both at 35 ($p = 0.004$) and 60 ($p = 0.004$) minutes after injection. $^{*}p > 0.05$ $^{**}p > 0.001$

Figure S2: Mean glucose blood levels. Both lean and obese groups had significantly reduced levels of mean blood glucose as a result of Exenatide both 35 (lean $p = 0.026$, obese $p = 0.007$) and 60 (lean $p = 0.030$, obese $p = 0.004$) minutes post injection compared with a pre-injection baseline. There were no significant differences between the lean and obese groups at any time point

Table S1: Blood levels of Exenatide